CLAIMS

We claim:

1. An inhibitor which is deactivatable by a reagent produced by a target cell comprising:

(a) a first moiety that binds, inhibits, suppresses, neutralizes, or decreases activity of a biologically active agent wherein said first moiety is operably linked to;

(b) a second moiety specifically cleavable by a reagent produced by a target cell, wherein said first and second moieties are not attached in nature and wherein specific cleavage of said second moiety causes reduction of binding, inhibiting, suppressing, or neutralizing activity of said inhibitor.

2. The inhibitor of claim 1, wherein said first moiety is selected from the group consisting of a peptide, a cyclic peptide, a polypetide, a peptidomimetic, a protein, a fusion protein, a hybrid molecule or a dimer, multimer, or a conjugate of the above.

3. The inhibitor of claim 1, wherein the first moiety is selected from the group consisting of a naturally occurring inhibitor, a soluble receptor, an antibody, a monoclonal antibody, a bispecific antibody, an antibody fragment, a single chain antibody, a peptabody, a peptide, a cyclic peptide, a peptide–lipid conjugate, a hormone, an antigen, an epitope, a receptor, a chemokine, a nucleic acid or a dimer, multimer, or a conjugate of the above.

4. The inhibitor of claim 1, wherein said inhibitor is selected from the group consisting of an antibody inhibitor, a monoclonal antibody inhibitor, a bispecific antibody inhibitor, a catalytic antibody inhibitor, a peptabody inhibitor, a receptor inhibitor, a soluble receptor inhibitor, a hormone inhibitor, a peptide inhibitor, a cyclic peptide inhibitor, a peptide–lipid conjugate inhibitor, a peptide–nucleic acid conjugate inhibitor, a nucleic acid/protein conjugate inhibitor, a delivery-enhancing transporter inhibitor, a pepducin inhibitor, a cytokine inhibitor, a chemokine inhibitor, a circularly permuted chemokine inhibitor, an interleukin inhibitor, an interferon inhibitor, or a dimer or multimer of the above.

5. The inhibitor of claim 1, wherein said active agent is selected from the group consisting of an antibody, a monoclonal antibody, a bispecific antibody, an antibody fragment, a single chain antibody, a peptabody, a diabody, a triabody, a peptide, a pepducin, a cyclic peptide, a peptide–lipid conjugate, a cell penetrating moiety, a membrane-tethering moiety, a nucleic acid, a hormone, an antigen, an epitope, a receptor, a chemokine, a cytokine, a circularly permuted chemokine, a circularly permuted cytokine, an interleukine, an interferone, a chemical drug, a component of a biological activation cascade, a coagulation system, a fibrinolysis system, a complement system, a kinin system, an enzyme which converts the inactive precursor of a pharmacological substance into the

pharmacologically active substance, a pharmacologically active substance, a coagulation factor selected from the group consisting of thrombin, factor Va, factor VIIa, factor IXa, factor Xa, TF coagulation-active fragments and factor XIIa; thrombin which is mutated in the region of the Arg-Thr cleavage site at amino acid position 327/328; a fibrinolytic protein selected from the group consisting of urokinase, tPA and functional hybrids thereof; a complement factor selected from the group consisting of CVF, C3b and functional cleavage products thereof; an antithrombotic protein selected from the group consisting of protein C, C-1S inhibitor, .alpha.1-antitrypsin, hirudin, AT-III, TFPI, PAI-1, PAI-2 and PAI-3; a kallikrein; a cytostatic, cytotoxic or inflammation-eliciting protein; an antiangiogenic protein; a heparinase; an immunomodulatory protein; an antiinflammatory protein; a protein which relieves damage to the nervous system; a protein which inhibits or neutralizes the neurotoxic effect of TNF.alpha.; an angiogenesis-stimulating protein; a hypotensive protein; an antiviral protein; a binding peptide or protein capable of specifically localizing to cells or tissues; a cytokine; an interferon; a tumor necrosis factor; oncostatin M or LIF; a cytokine receptor; the moiety of a cytokine receptor which is external to the cell; a cytokine antagonist; a growth factor; a growth factor receptor; the moiety of a growth factor receptor which is external to the cell; a chemokine; angiostatin; endostatin; platelet factor 4; TIMP-1, TIMP-2 or TIMP-3; a nitroreductase; a ß-glucuronidase; a carboxypeptidase; a ß-lactamase; a cytosine deaminase; a catalase; a peroxidase; a phosphatase; an oxidase; kallikrein or an endothelial cell nitric oxide synthase.

6. The inhibitor of claim 1 wherein at least one said second moiety is embedded within the first moiety.

7. The inhibitor of claim 1 wherein said first and second moieties are connected by a peptide, a lipid, a nucleic acid, a carbohydrate, a synthetic oligosaccharide analogue, a synthetic glycopeptide analogue, or a chemical linker.

8. The inhibitor of claim 1, wherein said second moiety is selected from the group consisting of a peptide, a polypetide, a lipid, a carbohydrate, a polysaccharide, a glycolipid, a nucleic acid, or a conjugate of the above.

9. The inhibitor of claim 1 wherein said second moiety is a peptide which comprises a sequence cleavable by a protease.

10. The inhibitor of claim 1 wherein the second moiety is selected from the group consisting of SKGSFSIQYTYHV (SEQ ID NO:2), HLGGSQQLLHNKQ (SEQ ID NO:3), SKGKGTSSQYSNTE (SEQ ID NO:4), DRVYIHPF (SEQ ID NO:5), VVCGERGFFYTP (SEQ ID NO:6), FFYTPKA (SEQ ID NO:7), KRRPVKVYP (SEQ ID NO:8), PVGKKRRPVKVY (SEQ ID NO:9),

KPVGKKRRPVKV (SEQ ID NO:10), GKPVGKKRRPVK (SEQ ID NO:11), TFAGNAVRRSVGQ (SEQ ID NO:12), PLGLWA (SEQ ID NO:13), PLFYS (SEQ ID NO:14), PRTLT (SEQ ID NO:15), or PLRLS (SEQ ID NO:16), HSSKLQ (SEQ ID NO:17), SQYSNT (SEQ ID NO:18), QFYSSNK (SEQ ID NO:19), VSQNYPIVQNFN (SEQ ID NO:20); SKARVLAEAMSN (SEQ.ID.NO:21), SIRKILFLDGIN (SEQ ID NO:22), SAPQVLPVMHPN (SEQ ID NO:23), SKTKVLWQPKN (SEQ ID NO:24), SKTKVLVVQPRN (SEQ ID NO:25), STTQCFPILHPN (SEQ ID NO:26); SGVVNASCRLAN (SEQ ID NO:27), SSYVKASVSPEN (SEQ ID NO:28), SALVNASSAHVN (SEQ ID NO:29), STYLQASEKFKN (SEQ ID NO:30), SSILNASVPNFN (SEQ ID NO:31), SQDVNAVEASSN (SEQ ID NO:32), SVYLQASTGYGN (SEQ ID NO:33), SKYLQANEVITN (SEQ ID NO:34), SELRTQSFSNWN (SEQ ID NO:35), SELWSQGIDDDN (SEQ ID NO:36), DLEVVTSTWVFN (SEQ ID NO:37), DEMEECASHLFN (SEQ ID NO:38), EDVVCCSMSYFN (SEQ ID NO:39), KGWRLLAPITAY (SEQ ID NO:40), SKPAKFFRLNFN (SEQ ID NO:41), SKPIEFFRLNFN (SEQ ID NO:42), SKPAEFFALNFN (SEQ ID NO:43), SLLKSRMVPNFN (SEQ ID NO:44), SLLIARRMPNFN (SEQ ID NO:45), SKLVQASASGVN (SEQ ID NO:46), SSYLKASDAPDN (SEQ ID NO:47), RPKPQQFFGLMN (SEQ ID NO:48), SLRPLALWRSFN (SEQ ID NO:49), SPQGIAGQRNFN (SEQ ID NO:50), DVDERDVRGFASFL (SEQ ID NO:51), SLPLGLWAPNFN (SEQ ID NO:52), SLLIFRSWANFN (SEQ ID NO:53), SGVVIATVIVIT (SEQ ID NO:54), SLGPQGIWGQFN (SEQ ID NO:55), KKSPGRVVGGSV (SEQ ID NO:56), PQGLLGAPGILG (SEQ ID NO:57), HGPEGLRVGFYESDVMGRGHARLVHVEEPHT (SEQ ID NO:58), GPQGLAGQRGIV (SEQ ID NO:59), GGSGQRGRKALE (SEQ ID NO:60), SLSALLSSDIFN (SEQ ID NO:61), SLPRFKIIGGFN (SEQ ID NO:62), SLPRFKIIGGFN (SEQ ID NO:63), SLLGIAVPGNFN (SEQ ID NO:64), FFKNIVTPRTPP (SEQ ID NO:65), QVVQLQNYDEED (SEQ ID NO:66), LPIFGESEDNDE (SEQ ID NO:67), QVVTGEAISVTM (SEQ ID NO:68), ALERTFLSFPTN (SEQ ID NO:69), KFQDMLNISQHQ (SEQ ID NO:70), Ala-Ala (SEQ.ID.NO.: 71) Ala-Ala-Pro-Val (SEQ.ID.NO.: 72), Ala-Ala-Met (SEQ.ID.NO.: 73) Ala-Ala-Pro-Phe (SEQ.ID.NO.: 74), Ala-Ala-Pro-Met (SEQ.ID.NO.: 75), Ala-Ala-Arg (SEQ.ID.NO.: 76) Ser-Ala-Ala-Arg (SEQ.ID.NO.: 77), Ser-Ser-Ala-Ala-Arg (SEQ.ID.NO.: 78), Ser-S carboxyl sugar-Ala-Ala-Arg (SEQ.ID.NO.: 79), Ala-Ala-Asp (SEQ.ID.NO.: 80), Ser-Ala-Ala-Asp (SEQ.ID.NO.: 81), Ser-Ser-Ala-Ala-Asp (SEQ.ID.NO.: 82), Arg-Pro-Lys-Pro-Leu-Ala-Nva (SEQ.ID.NO.: 83), Ser-Arg-Pro-Lys-Pro-Leu-Ala-Nva (SEQ.ID.NO.: 84), Ser-Ser-Arg-Pro-Lys-Pro-Leu-Ala-Nva (SEQ.ID.NO.: 85), Pro-Cha-Gly-Nva-His-Ala-Dpa-NH.sub.2 (SEQ.ID.NO.: 86), Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH.sub.2 (SEQ.ID.NO.: 87), Pro-Cha-Gly-Nva (SEQ.ID.NO.: 88), Pro-Leu-Gly-Leu (SEQ.ID.NO.: 89), Gly-Pro-Arg (SEQ.ID.NO.: 90), Leu-Pro-Arg (SEQ.ID.NO.: 91), Glu-Gly-Arg (SEQ.ID.NO.: 92), Gly-Pro-Gln-Gly-Ile (SEQ.ID.NO.: 93),

or to a peptide of 20 or fewer amino acids in length, wherein the sequence comprises the amino acids: X.sub.5 X.sub.4 X.sub.3 X.sub.2 X.sub.1,

wherein X.sub.5 is from 0 to 16 amino acids; X.sub.4 is serine, isoleucine, or lysine; X.sub.3 is serine or lysine; X.sub.2 is leucine or lysine; and X.sub.1 is glutamine, asparagine or tyrosine; or to a oligopeptide that comprises an amino acid sequence selected from: AsnLysIleSerTyrGlnSer (SEQ.ID.NO.: 94), LysIleSerTyrGlnSer (SEQ.ID.NO.: 95), GlyGluAsnGlyValGlnLysAspValSerGlnXaaSerIleTyrSerGlnThrGlu (SEQ.ID.NO.: 96), GlyLysGlyIleSerSerGlnTyrSerAsnThrGluGluArgLeu (SEQ.ID.NO.: 97), AsnLysIleSerTyrTyrSer (SEQ.ID.NO.: 98), AsnLysAlaSerTyrGlnSer (SEQ.ID.NO.: 99), SerTyrGlnSerSer (SEQ.ID.NO.: 100), hArgTyrGlnSerSer (SEQ.ID.NO.: 101), AsnLysIleSerTyrGlnSerAla (SEQ.ID.NO.: 102), AlaAsnLysIleSerTyrTyrSer (SEQ.ID.NO.: 103), AlaAsnLysAlaSerTyrGlnSer (SEQ.ID.NO.: 104), SerTyrGlnSerSerThr (SEQ.ID.NO.: 105), SerTyrGlnSerSerSer (SEQ.ID.NO.: 106), LysTyrGlnSerSerSer (SEQ.ID.NO.: 107), hArgTyrGlnSerSerSer (SEQ.ID.NO.: 108), SerTyrGlnSerSerLeu (SEQ.ID.NO.: 109), wherein hArg is homoarginine and Xaa is any natural amino acid; or a combination thereof.

11. The inhibitor of claim 1 wherein said reagent is selected from the group consisting of a protease, a lipase, a nuclease, or a glycolytic enzyme.

12. The inhibitor of claim 1 wherein said reagent is selected from the group consisting of a prostate specific antigen, a human prostate-associated protease, a matrix metalloproteinase, a plasminogen activator, a cathepsin, a urokinase, a neutrophil elastase, a calpain, a urokinase-type plasminogen activator, a tissue-type plasminogen activator, a kallikrein, a viral protease, a fungal protease, a bacterial protease, a parasitic protease, a protease secreted by a cancer cell, a matriptase, a Kunitz-type serine protease, a thiol-dependent protease, a Plasmondium falciparum protease, a Candida acid protease, a human cytomegalovirus protease, a human herpes virus protease, a varicella zoster virus protease, a hepatitis A virus protease, a hepatitis C virus protease, a Epstein-Barr virus-specific protease, an infectious laryngotracheitis virus protease, a catalytic RNA, or a catalytic antibody.

13. The inhibitor of claim 1 wherein said reagent is produced by endothelial cells, cells adjoining activated endothelial cells, activated or proliferating endothelial cells, tumor cells, muscle cells, smooth muscle cells, fibroblasts, macrophages, lymphocytes, liver cells, kidney cells, synovial cells, joint cells, inflammatory cells, virus-infected cells, bacteria-infected cells, parasite-infected cells, bronchial epithelial cells, glia cells, or leukemia cells.

14. The inhibitor of claim 1 wherein the inhibitor alone or together with an active agent is in a biodegradable, biocompatible polymeric delivery material, in a slow release implant, in a microencapsulated composition, or a conjugate with a biodegradable polymer.

15. The inhibitor of claim 1 which further comprises or associates with a recognition domain that binds to a target structure, an exterior surface of a targeted cell, a cell surface marker, an extracellualr matrix, or components thereof.

16. The inhibitor of claim 15, wherein said recognition domain binds to activated or proliferating endothelial cells, to tumor cells, to muscle cells, smooth muscle cells, to prostate cells, to fibroblasts, to macrophages, to lymphocytes, to liver cells, to kidney cells, to synovial cells, to joint cells, to blood vessels, to inflammatory cells, to virus-infected cells, to bronchial epithelial cells, to glia cells, or to leukemia cells.

17. The inhibitor of claim 15, wherein said recognition domain is selected from the group consisting of an antibody, a monoclonal antibody, a bispecific antibody, an antibody fragment, a single chain antibody, a peptabody, a peptide, a cyclic peptide, a peptide–lipid conjugate, a hormone, an antigen, an epitope, a receptor, a chemokine, a cytokine, a circularly permuted chemokine, a circularly permuted cytokine, an interleukine, an interferone, a tissue factor, or compositions and variants thereof.

18. A method of site specific activation of an active agent comprising administration of an inhibitor which is deactivatable by a reagent produced by a target cell comprising:
(a) a first moiety that binds, inhibits, suppresses, neutralizes, or decreases activity of a biologically active agent wherein said first moiety is operably linked to;
(b) a second moiety specifically cleavable by a reagent produced by a target cell, wherein said first and second moieties are not attached in nature and wherein specific cleavage of said second moiety causes reduction of binding, inhibiting, suppressing, or neutralizing activity of said inhibitor and restoration of activity of said active agent; said inhibitor is administered alone or together with an active agent such that the activity of the active agent is reduced until it reaches a target cell producing a reagent wherein the inhibitor is cleaved by said reagent and activity of said active agent is restored.

19. The method of claim 18, wherein said administration to a vertebrate has a desired treatment effect.

20. A method for treating a cancer cell comprising contacting the cell with an inhibitor which is deactivatable by a reagent produced by a target cell comprising:
(a) a first moiety that binds, inhibits, suppresses, neutralizes, or decreases activity of a biologically active agent wherein said first moiety is operably linked to;
(b) a second moiety specifically cleavable by a reagent produced by a target cell, wherein said first and

second moieties are not attached in nature and wherein specific cleavage of said second moiety causes reduction of binding, inhibiting, suppressing, or neutralizing activity of said inhibitor and restoration of activity of said active agent; said inhibitor is administered alone or together with an active agent such that the activity of the active agent is reduced until it reaches a target cell producing a reagent wherein the inhibitor is cleaved by said reagent and activity of said active agent is restored.

21. The method of claim 20 wherein said inhibitor is administered in a composition comprising a biologically effective amount of the inhibitor alone or together with an active agent and a pharmaceutically acceptable carrier, diluent or excipient to a vertebrate.

22. The method of claim 20 wherein said cancer cell is selected from the group consisting of a colon cancer, a prostate cancer, a breast cancer, a T-cell or B-cell lymphoproliferative disease, a cancer cell expressing a plasma membrane tyrosine kinase receptor, a head and neck cancer, a squamous cell carcinoma, a gastrointestinal cancer, a non small cell lung cancer, a melanoma, a kidney cancer, an ovarian cancer, or a pancreatic cancer cell.

23. The method of claim 20 wherein said treatment inhibits growth of a cancer in a vertebrate.

ABSTRACT

The invention relates to molecules inhibiting biologically active componds and further comprising moieties specifically cleavable by a reagent produced by a target cell. More specifically, the invention relates to inhibitors that bind, inhibit, suppress, neutralize, or decrease activity of a biologically active agent. Inhibitors comprise at least one moiety that bind, inhibit, suppress, neutralize, or decrease activity of a biologically active agent and at least one moiety that can be cleaved specifically by a reagent produced by target cells. The cleavage deactivates the inhibitor. Following cleavage, the active agent is liberated into the local environment. Administration of the inhibitor alone or together with the active agent suppress the compound's activity until it reaches the proximity of a target cell. This targeted specific release enables the agent concentration in a site to reach levels that have desired therapeutic effects without systemic toxicity. The invention also relates to production and uses of the inhibitors in diagnosis and treatment of a disease.

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